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# EVALUATION OF *PSEUDOMONAS FLUORESCENS* AGAINST BROWN SPOT, SHEATH BLIGHT AND GLUME DISCOLOURATION OF RICE

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***ABSTRACT***

***Pseudomonas fluorescens is the most widely used biocontrol agent for crop disease management. Field studies were conducted to evaluate Pseudomonas fluorescens against sheath blight, brown spot and glume discolouration of rice. Different combinations of application methods viz, seed treatment + seedling root dip, seed treatment + soil application, seed treatment + seedling root dip + foliar spray, seed treatment + soil application + foliar spray were evaluated in the field in comparison with fungicide carbendazim. The application of Pseudomonas fluorescens by any of these four combinations reduced the severity of brown spot, sheath blight and glume discolouration as effective as the fungicide, carbendazim and were significantly superior to control. The combination of three applications reduced sheath blight severity significantly than two applications. There was significant improvement in grain yield also by the application of Pseudomonas fluorescens. Different combinations of applications of P. fluorescens suitable to the crop establishment method can be selected for disease management in rice.***

***KEYWORDS: Biological Control, Pseudomonas Fluorescens, Sheath Blight, Brown Spot, Glume Discolouration, Rice***

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Original Article

## INTRODUCTION

Rice is one of the major crops cultivated in India. It occupies 34 per cent of the total cultivated area of the nation. In the world scenario, India ranks first in area and second in rice production. With the developments in rice production technology, significant changes have occurred in the traditional system of rice cultivation. With the introduction of high yielding fertilizer responsive varieties and their wide spread cultivation resulted in associated problems of occurrence of pests, diseases and weeds. Eventhough several varieties with resistance to certain pests and diseases have been released from different parts of the world and from India, the host plant resistance alone is not sufficient to manage biotic constraints. The main reason for this is lack of resistance to many pests and diseases as well as changes in population of pathogen and pests happened over time. Among the diseases of rice, blast, sheath blight, and bacterial blight are major problems throughout the rice growing countries. The most viable and economic strategy of disease management is integrated approach comprising biological control, host

Plant resistance, cultural control and chemical control. Considering the environmental and health hazards of indiscriminate and long time use of chemicals, non chemical methods of disease management including

botanical formulations and biological control agents is gaining importance worldwide. In a crop like rice, where maximum pesticides are being used, such environment friendly practices are very important by which the health hazards and the risk of contamination of chemicals in the ecosystem can be reduced directly. The biological control agents are widely being used in crop disease management. The success of biological control of crop disease depends mainly on the efficacy of the biocontrol agents against pathogens, delivery systems and crop management practices followed.

In the state of Kerala the important fungal diseases of rice are blast, sheath blight, brown spot and false smut and the bacterial disease is bacterial blight. Eventhough these diseases are seen throughout the year these are mainly seen in *kharif s*eason. Among the fungal diseases, leaf blast is restricted mainly to uplands and certain ecosystems and varieties. Sheath blight is widely seen and no varieties are resistant to this disease. Brown spot is another fungus which was a minor disease earlier and is becoming a major problem in recent years. The post flowering diseases were not damaging so far. But in the last few years, false smut and glume discolouration are causing yield losses in farmers' field. Several fungi are reported to be associated with glume discolouration. Association of pathogens namely *Bipolaris oryzae, Alternaria alternata, Alternaria padwickii, Drechslera oryzae, Fusarium moniliforme, Curvularia oryzae, Nigrospora oryzae* and *Aspergillus niger* was reported from Pakistan (Arshad *et al*., 2009). Bhat *et al.,* (2009) found out the association of eight fungi namely *Helminthosporium oryzae, Curvularia lunata, Alternaria* spp, *Rhizoctonia solnai, Trichothecium* spp, *Aspergillus niger, Pyricularia grisea* and *Fusarium graminearum* with glume discolouration in rice of which *Helminthosporium oryzae, Curvularia lunata* and *Alternaria* spp were predominant ones. Glume discolouration as a major problem was reported from Bangladesh by Mia *et al.,* (1996).

Use of fungicides is the major practice of disease management in the present day rice production system. Biological control is slowly spreading in rice cultivation in the state. The use of biological control agents in rice for the management of various diseases of rice has been studied by various workers. *Pseudomonas fluorescens* is one of the most widely used biocontrol agent for crop disease management. In addition to the biological control of plant pathogens these are reported to be having growth promoting ability. The success of biological control of sheath blight of rice using *Pseudomonas fluorescens* has been reported by earlier workers. (Rabindran *et al.*,1996; Nandakumar *et al.,* ; 2001Commare *et al.,* 2002; Singh *et al.*, 2005; Tiwari *et al.,* 2009).

*Pseudomonas fluorescens* was found effective for the management of brown spot (Kumar *et al.,* 2014) and sheath rot (Saravankumar *et al.,* 2009). The efficacy of antimicrobial metabolites of one strain of *Pseudomonas fluorescens* against rice fungal pathogens *Magnaportha grisea, Drechslera oryzae, Rhizoctonia solani* and *Sarocladium oryzae* was reported by Reddy *et al.,* (2008). A study was conducted to evaluate five isolates of *Pseudomonas fluorescens* against bacterial blight of rice (Gangwar, 2013). Two isolates Pf 83 and FLP 84 isolated from rice phylloplane reduced the disease severity and increased yield in two seasons. Prophylatic sprays of *Pseudomonas fluorescens* strain MBPF – 01 or spray of nano copper after pathogen inoculation of bacterial leaf blight followed by spraying of *Pseudomonas fluorescens* reduced the bacterial blight intensity (Mondal *et al.,* 2010).

The biological control of diseases in rice by *P. fluorescens* is achieved through different means. Induction of systemic resistance in plants is one of the most important mechanisms of disease control. *Pseudomonas fluorescens* induced systemic resistance in rice against blast (Vleesschauwer *et al.,* 2008) and bacterial blight (Vidhyasekaran *et al.,* 2001) has been reported.

The present study was undertaken with the objective of evaluation of biocontrol agents for the management of rice diseases in wetland rice ecosystem of Kerala state of India. The diseases, sheath blight, brown spot and glume discolouration naturally occurring in the field were studied.

## MATERIALS AND METHODS

### Biocontrol Agents

Native isolate of *Pseudomonas fluorescens* proven efficient for disease management developed at the College of Agriculture, Vellayani, Kerala was used for the study. The isolate is being used for commercial production system in the state. *Pseudomonas fluorescens* was multiplied in king's B medium and was formulated using talc as carrier. The talc based formulation containing colony forming unit (cfu) of $1 \times 10^8$ $g^{-1}$ was used for the study.

### Evaluation of Pseudomonas fluorescens against Rice Diseases

Four field experiments were conducted in rainfed lowland system, during the years 2013 -2014 at the Regional Agricultural Research Station, Pattambi at Kerala Agricultural University.

Treatments

**$T_1$:** *Pseudomonas fluorescens* seed treatment (ST) (10g $kg^{-1}$ seed) + seedling root dip (SD) (20 g $l^{-1}$)

**$T_2$.** Pseudomonas *fluorescens* seed treatment (10g $kg^{-1}$ seed) + soil application (SA) (2.5 kg $ha^{-1}$)

**$T_3$:** *Pseudomonas fluorescens* seed treatment (10g $kg^{-1}$ seed) + seedling root dip (20 g $l^{-1}$) + foliar spray (10 g $l^{-1}$)

**$T_4$:** *Pseudomonas fluorescens* seed treatment (10g $kg^{-1}$ seed) + soil application (2.5 kg $ha^{-1}$) + foliar spray (FS) (10 g $l^{-1}$)

**$T_5$:** Carbendazim foliar spray (1 g $l^{-1}$)

**$T_6$:** Control (Untreated check)

### Treatment Application

**Seed Treatment** - The required quantity of seeds were soaked in water mixed with *Pseudomonas fluorescens* (10g per litre of water). Seeds were soaked for 12 hours. After that drained out and kept for germination in gunny bags. Water was sprinkled on it daily and on third day germinated seeds were sown in the nursery field.

**Seedling Root Dip** – Seedlings were pulled out from the nursery and the roots were dipped in water mixed with *Pseudomonas fluorescens* (20g $l^{-1}$) for one hour, before transplanting.

**Soil Application** - 2.5 kg of talc based formulation was mixed with farmyard manure (50 kg $ha^{-1}$) and broadcasted in the field one week after transplanting.

**Foliar Spray of *Pseudomonas Fluorescens*** - Foliar spray of *P. fluorescens* ( 10 g $l^{-1}$) was given one month after transplanting.

**Foliar Spray of Fungicide** - Foliar spray of carbendazim (1 g $l^{-1}$) was given at tillering and panicle initiation stage.

The experiment was laid out in randomized block design with four replications. The susceptible variety Jyothi was used for the study. Seedlings were raised in the nursery and 25 days old seedlings were transplanted in the main field at a spacing of 15 × 10 cm in plots of size 10 $m^2$.

**Disease Recording**

Observations on percentage severity of brown spot, sheath blight and glume discolouration were recorded. The data was analysed using MSTATC programme. The analysis of variance was performed and means were separated by Fischer's LSD Test.

## RESULTS AND DISCUSSIONS

The data on severity of various diseases is given in Table 1. There was a significant difference among the treatments on severity of brown spot, sheath blight and glume discolouration. The application of *P. fluorescens* in any of the combinations as seed treatment + seedling root dip, seed treatment + soil application, seed treatment + seedling root dip + foliar spray or seed treatment + soil application + foliar spray reduced the brown spot severity significantly compared to control. The prophylatic application of *Pseudomonas fluorescens* was found to be effective as two foliar sprays of fungicide, carbendazim in reducing the brown spot severity.

The sheath blight severity recorded in all the treatments were significantly less compared to control. It was lowest when three applications of *Pseudomonas fluorescens* was given either as ST + SD + FS - $T_3$ (18.72 %) or ST + SA + FS - $T_4$ (18.94 %). The glume discolouration in all the *Pseudomonas fluorescens* applied fields were significantly less than that of control and were equally effective as the fungicide application. The yield recorded in all the treatments was significantly higher than that of control (Table 1).

The field evaluation of biocontrol agents against diseases of rice done so far were mainly on blast sheath blight and bacterial blight. Since the diseases like brown spot and glume discolouration are becoming major problems, the information on the efficacy of the biocontrol agents against these diseases also would be helpful for disease management in rice. The management of rice diseases by the use of biocontrol agent *Pseudomonas fluorescens* has been reported by various workers. Among the eight phylloplane microorganisms tested, *Cladosporium* spp, *Penicillium* spp, *Aspergillus* spp, and *Pseudomonas* spp. recorded inhibition of 62.15, 50.34, 49.20 and 47.24 respectively against *Helminthosporium oryzae* (Harish *et al.,* 2007). Even though the *in vitro* antagonism of the biocontrol agent *P. fluorescens* against brown spot pathogen was reported by few workers (Reddy *et al.,* 2008; Arumughan *et al.,* 2013) in field conditions, the control of brown spot by *P. fluorescens* has rarely been reported (Kumar *et al.,* 2014; Kumar *et al,,* 2012). The control of brown spot by seed treatment with *Pseudomonas fluorescens* and *Pseudomonas synxatha* under pot culture condition was obtained in a study conducted by Ludwig *et al.,* (2009). In the present study a reduction of brown spot by 12-18 per cent was resulted by application of *P. fluorescens* compared to control (Figure 1).

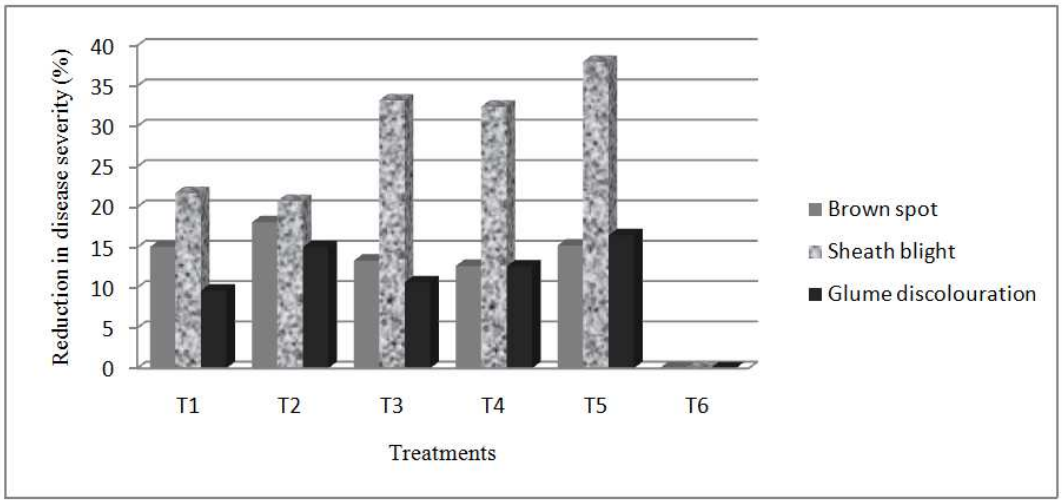


**Figure 1: Effect of *Pseudomonas fluorescens* on Diseases of Rice**

The application of *P. fluorescens* reduced sheath blight severity by 21 – 38 per cent in the field (Figure 1). It was comparable to that of application of fungicide, carbendazim. The reduction in sheath blight pathogen by *P. fluorescens* under *in vitro* (Tiwari *et al*., 2009 ; Reddy *et al.,* 2008) and in the field (Nandakumar *et al.,* 2001; Singh & Sinha, 2005; Rabindran & Vidyasekaran, 1996; Commare *et al.,* 2002) was reported. Three applications ST + SA + FS or ST + SD + FS reduced the sheath blight severity significantly compared to two applications. Vidyasekaran and Muthamilan (1994) studied the efficacy of powder formulation of *P. fluorescens* for the control of sheath blight of rice. Among the different methods of application tested they obtained reduction in sheath blight severity and increase in yield when seed treatment was combined with root dip and soil application or seed treatment combined with root dip, soil application and foliar spray compared to single application. Singh and Sinha (2005) also obtained the same results revealing that more than two applications were more effective against sheath blight.

There was a reduction of 9.62 - 15.03 per cent reduction in severity of glume disolouration obtained in the field due to the application of *Pseudomonas fluoresens* (Figure1). The glume discolouration of rice is a relatively newer disease in this region of country. Very few reports are available on this disease. Fungicidal management of glume discolouration was reported by Anwar and Bhat (2008). They got control of glume discolouration by the fungicide carbendazim and mancozeb. The control of glume discolouration by the antagonistic micro organisms was studied by Akila and Ebenezar (2009).They could obtain the reduction in glume discolouration by spraying of *P. fluorescens* in pot culture. The bioagents were evaluated against *Curvularia lunata* the fungi associated with grain discoloration *in vitro* by Sumangala *et al.,* (2008). The biocontrol agents *Bacillus subtilis*, *Trichoderma viride* and *Trichoderma harzianum* found effective in inhibiting the mycelial growth of the pathogen. The highest germination percentage was recorded when seeds were treated with *Trichoderma viride*.

The yield recorded in different treatments with *P. fluorescens* was significantly superior to control (Table1). There was an improvement of 9.75 to 13.86 per cent in yield due to the application of *P. fluorescens* (Figure 2). The use of *Pseudomonas fluorescens* not only manage the disease but also promotes the growth and thereby improves the yield (Gangwar, 2013). The biological control of pathogens is achieved through various mechanisms of actions such as induced systemic resistance, antibiosis and siderophore production. The induction of systemic resistance in rice and thereby the reduction in bacterial blight severity was reported by Vidhyasekaran *et al*. (2013).

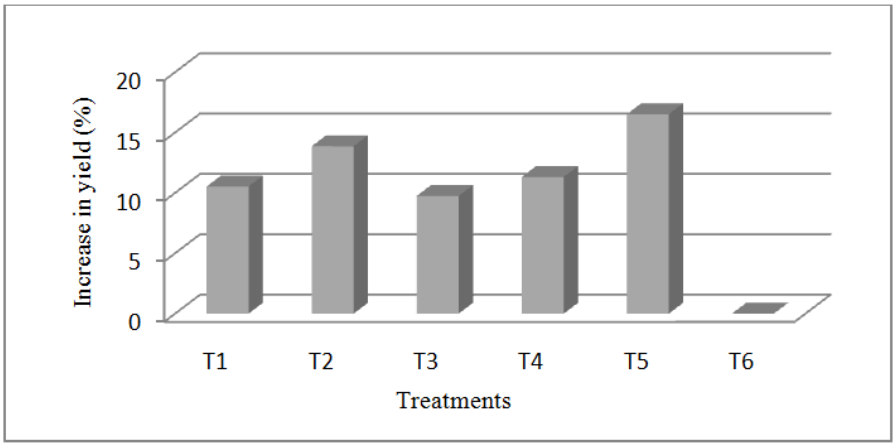


**Figure 2: Effect of *Pseudomonas fluorescens* on Grain yield of Rice**

## CONCLUSIONS

The application of *Pseudomonas fluorescens* twice as seed treatment + soil application or seed treatment + seedling root dip or thrice as seed treatment + soil application + foliar spray or seed treatment + seedling root dip+ foliar spray reduced the severity of brown spot, sheath blight and glume discolouration in rice significantly compared to control and comparable to that of application of fungicides carbendazim twice. The delivery systems of biocontrol agent can be chosen to suit with the crop establishment methods. Here mainly two sets of application of *P. fluorescens* were tested, seed treatment + soil application, seed treatment + soil application + foliar spray and seed treatment + seedling root dip or seed treatment + seedling root dip + foliar spray. For direct seeding system the first set including soil application will be suitable whereas in transplanting system, either of these can be followed.

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## APPENDICES

**Table 1: Effect of Biocontrol Agents on Brown Spot, Sheath Blight and Glume Discolouration of Rice**

| Treatments | Brown Spot Severity (%) | Sheath Blight Severity (%) | Glume Discoloration Severity (%) | Yield (kg ha$^{1}$) |
|---|---|---|---|---|
| | 33.88 (33.64)$^{b}$ | 21.94 (26.86)$^{b}$ | 32.21 (33.55)$^{b}$ | 4790$^{a}$ |
| $T_2$ | 32.66 (33.19)$^{b}$ | 22.22 (26.84)$^{bc}$ | 30.28 (32.19)$^{bc}$ | 4928$^{a}$ |
| $T_3$ | 34.56 (34.43)$^{b}$ | 18.72 (24.08)$^{d}$ | 31.84 (33.30)$^{b}$ | 4750$^{a}$ |
| $T_4$ | 34.82 (35.19)$^{b}$ | 18.94 (24.84)$^{cd}$ | 31.14 (32.89)$^{bc}$ | 4818$^{a}$ |
| $T_5$ | 33.81 (34.65)$^{b}$ | 17.38 (23.63)$^{bc}$ | 29.76 (31.86)$^{c}$ | 5043$^{a}$ |
| $T_6$ | 39.88 (38.94)$^{a}$ | 28.03 (30.99)$^{a}$ | 35.64 (36.07)$^{a}$ | 4328$^{b}$ |
| CD (0.05%) | 2.05 | 1.79 | 1.41 | 392 |

Values in parenthesis are arscine transformed. Each value is pooled mean of four trials each with four replications. In the same column means followed by the same letter are not significantly different (P= 05) according to Fischer's LSD test.